Ichthyological note – *Note ichtyologique*

Received: 14 Mar. 2016
Accepted: 6 Jun. 2016
Editor: R. Causse

Unambiguous identification of the non-indigenous species *Cynoscion regalis* (Sciaenidae) from Portugal

by

Philippe BÉAREZ* (1), Sónia GABRIEL (2, 3) & Agnès DETTAI (4)

Résumé. – Premier signalement d'un sciénidé exotique, *Cynoscion regalis*, sur la côte du Portugal.

Plusieurs spécimens de *Cynoscion regalis* ont été capturés dans l'estuaire du Sado, côte ouest du Portugal. Cette espèce est un habitant commun des eaux côtières et estuariennes de la façade atlantique de l'Amérique du Nord. Ce nouveau signalement au Portugal, à environ 5000 km à l'est de leur aire de répartition, est surprenant mais l'identification a été confirmée par des caractères morphométriques et la séquence du gène CO1. Cette découverte récente pourrait être due au transport par bateau, la principale voie d'invasion pour les poissons marins exotiques.

Key words. – Sciaenidae – *Cynoscion regalis* - Portugal - New record - Alien species - DNA barcoding.

Croakers or drums (Sciaenidae) are widely distributed in the shallow coastal, estuarine tropical and subtropical waters of all oceans. Many species are of considerable commercial value and are often linked to economically important fisheries. However, due to overharvesting and habitat degradation populations of sciaenids have decreased in many areas (Chao *et al*., 2015), and some large species are close to extinction: *e.g. Totoaba macdonaldi* (Gilbert, 1890) and *Bahaba taipingensis* (Herre, 1932). This situation led to the development of dedicated aquaculture programs, and several members of the sciaenid family are now cultured on a large scale: *e.g. Sciaenops ocellatus* (Linnaeus, 1766), *Argyrosomus regius* (Asso y del Rio, 1801), *Larimichthys crocea* (Richardson, 1846) and *Larimichthys polyactis* (Bleeker, 1877).

In the Northeast Atlantic Ocean and the Mediterranean Sea, sciaenids are represented by five species (Trewavas, 1979; Chao, 1986): the meagre *Argyrosomus regius*; the brown meagre *Sciaena umbra* Linnaeus, 1758; the Canary drum *Umbrina canariensis* Valenciennes, 1843; the shi drum *Umbrina cirrosa* (Linnaeus, 1758); and the fusca drum *Umbrina ronchus* Valenciennes, 1843. All five of these species occur in Portuguese coastal waters (Carneiro *et al*., 2014).

As far as we know, exotic species of sciaenids have rarely been found in the Northeast Atlantic: all have originated in North America. Stevens *et al*. (2004) reported the presence of two specimens (caught in 1998 and 2001) of *Micropogonias undulatus* (Linnaeus, 1766) in Belgium, and declared its occurrence was most likely to be due to transportation in ship ballast water. More recently, Acosta *et al*. (2013) reported the 2011 discovery of an individual *Cynoscion nebulosus* (Cuvier, 1830) from the Gulf of Cadiz, Spain. Golani *et al*. (2015) reported on an individual *Sciaenops ocellatus* (Linnaeus, 1766), caught in 2011, off the Mediterranean coast of Israel.

Here, we report on the first recorded Northwest Atlantic species *Cynoscion regalis* (Bloch & Schneider, 1801) from Portuguese and European waters.

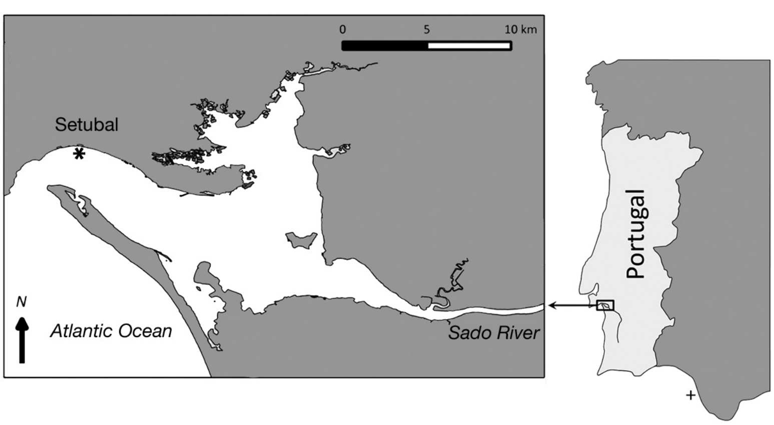

Figure 1. – Map of the area showing localities of captured *Cynoscion regalis*: (*) in Setubal, Portugal, 2015 ; (+) in the Gulf of Cadiz, Spain, 2011.

MATERIAL AND METHODS

Three specimens were obtained from leisure fishermen in the Port of Setubal, Sado Estuary, Portugal (38°31'14"N-8°53'15"W) on the 1st of October 2015 (Fig. 1). The fishermen, who caught several specimens (7) with a hook and line from the pier of the harbour, declared the species unknown to them and gave us part of their catch. The following day several other specimens of a similar size were observed at the Port of Setubal fish market (G. Piquès, comm. pers.).

Identification was corroborated using sequence data. DNA was extracted as per Winnepenninckx *et al*. (1993). The partial sequence of the mitogenome was obtained using long PCR's and a two level mul-

(1) Archéozoologie, archéobotanique : sociétés, pratiques et environnements (UMR 7209), Sorbonne Universités, MNHN, CNRS, Muséum national d'Histoire naturelle, 55 rue Buffon, CP 56, 75005 Paris, France.
(2) Archaeosciences Laboratory (LARC), Direcção Geral do Património Cultural (DGPC), Rua da Bica do Marquês 2, 1300-087 Lisbon, Portugal. [sgabriel@dgpc.pt]
(3) Research Center in Biodiversity and Genetic Resources (CIBIO), University of Porto, Portugal.
(4) Institut de Systématique, Évolution, Biodiversité ISYEB (UMR 7205), Sorbonne Universités, CNRS, MNHN, UPMC, EPHE, Muséum national d'Histoire naturelle, 57 rue Cuvier, CP 30, 75005 Paris, France. [adettai@mnhn.fr]
* Corresponding author [bearez@mnhn.fr]

tiplex approach following the protocol described in Hinsinger *et al.* (2015), using primer pairs MtL11910-12SH1478 and an overlapping 12SL1091-MtH7061. The sequences were assembled and checked using Geneious 7.1.7 (Biomatters Ltd.). Annotation was performed using MitoAnnotator (Iwasaki *et al.*, 2013) and compared with other published mitogenomes. All nucleotide changes and indels were checked against the assembly and compared to the closest available mitogenomes and sequences. The sequence for the partial (barcode region) cytochrome oxidase 1 (CO1) was used to BLAST search the full Barcode of Life Database (BOLD) and the GenBank. The available sequence markers for the *Cynoscion* species (Cytochrome b, control region, 12S rDNA, 16S rDNA) were individually chosen to BLAST search the GenBank. The new partial mitogenome sequence was deposited in the GenBank Nucleotide database under accession number KX364159. The individual mitochondrial markers were deposited in BOLD with the specimen information under reference PRFIS001-16. The proposed standards for mitogenome metadata (Strohm *et al.*, 2015) were rigorously followed.

The voucher specimen was deposited in the zoological collection of the Museu Nacional de História Natural e da Ciência da Universidade de Lisboa (Portugal): reference number MB06-005523. A second specimen was deposited in the Laboratório de Arqueociências, Direcção Geral do Património cultural collection: reference number CIPA2688. The third specimen was discarded after dissection, though the tissue samples and the extracted otoliths (sagittae) are now preserved in the MNHN collection: reference number MNHN-ICOT-01951.

RESULTS

Both morphometric (cf. Chao, 2003) and molecular data confirm that the sciaenid specimens from Portugal belongs to the species *Cynoscion regalis* (Fig. 2).

Morphologic data. – 235-243 mm TL; 198-204 mm SL; 60-62 mm head length; 130-140 g total weight. Fusiform body, slightly compressed. Mouth large, upper jaw with two strong canine-like teeth posteriorly oriented. Ctenoid body scales. Dorsal-fin rays X+I, 25; anal-fin rays II, 11. Pectoral fin slightly longer than pelvic fin. Dorsal and anal fins with scaly basal sheath. Caudal fin slightly rhomboidal. Horn-like appendages arising anteriorly from the gas bladder. Sagitta oval and elongate, with ventral and dorsal rims slightly curved and ornamented (Fig. 3). Body with numerous small black spots on back, forming not well defined undulating lines, that do not extend onto the fins.

The individuals appeared to be immature, which is in agreement with Shepherd and Grimes (1984) who stated that maturity is attained at about 250 mm TL.

Molecular data. – The partial mitogenome sequence was 11648 bp long, with an average coverage of 28.2. It completely covers ND5, ND6, tRNA-Glu, cytochrome b, tRNA-Thr, tRNA-Pro, control region, tRNA-Phe, 12S rDNA, tRNA-Val, 16SrDNA, tRNA-Leu, ND1, tRNA-Ile, tRNA-Gln, tRNA-Met, ND2, tRNATrp, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, and cytochrome oxidase 1; arranged in the classical teleost mitochondrial gene order (Miya and Nishida, 2015). The CO1 sequence is identical to the nine sequences for *C. regalis* present in BOLD. The cluster contained only sequences for this species. For 12S and 16S our sequences were identical or with only a single difference, compared to the partial and only sequences available for the species in the sequence database. For the controlled region, the single sequence present for *C. regalis* in Genbank (DQ179650.1) is the closest match at 98% similarity. For all markers, distance trees place our sequences unambiguously with *C. regalis*. The molecular data, therefore, strongly supports the morphological identification.

Moreover, these are the first sequences available for any *Cynoscion* species for ND1, ND2, ND5, ND6; and include 14 types of tRNAs for this species. As such, they are a useful reference for future phylogenetic studies or environmental DNA monitoring.

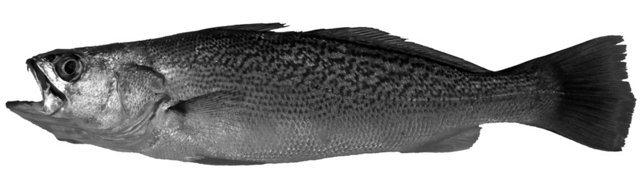

Figure 2. – *Cynoscion regalis*, MB06-005523, 235 mm TL, Setubal, Portugal.

DISCUSSION

Cynoscion is an amphi-American genus (Sasaki, 1989) with 24 recognized valid species: 12 from the Eastern Pacific and 12 from the Western Atlantic (Chao, 2003). From amongst the latter, *Cynoscion regalis* is probably the most cold-water tolerant, being distributed along the North American coast from Florida to Cape Cod. Therefore, the discovery of a *Cynoscion* species 5,000 km east of their known distribution area is puzzling.

Over the last few decades, the number of non-native species occurring in areas far from their original range has increased significantly. This is especially true in the Mediterranean Sea where Lessepsian and Atlantic migrants are more numerous (Golani *et al.*, 2007). In these cases, natural corridors constitute clearly identified pathways of invasion. In the Eastern Atlantic, most of the fish migrants go either north or south, as the geographic distribution boundaries of species move with global warming. This cannot be the case for *Cynoscion regalis*: a North American species that had to cross the Atlantic (ca. 5,000 km) to arrive on the Portuguese coast. An extension of its range by natural dispersal, via larval transport

Figure 3. – Left (lateral view) and right (mesial view) sagittae of *Cynoscion regalis*, MNHN-ICOT-01951. Scale bar = 1 cm.

using the Gulf Stream and the Azores Current, could explain the occurrence of the species in Portugal. However, the reproductive biology of this species does not support such a hypothesis. They spawn either in the muddy or sandy bottoms of estuaries, or close to the mouths, at a water depth of no more than 5 to 10 meters (Welsh and Breder, 1924); and though their fertilized eggs are buoyant and can be carried by the tidal currents, they seem to sink before hatching (*i.e*. before 1-2 days). In addition, the duration of the pelagic larval phase is short, with larval and juvenile development generally occurring in estuarine nursery zones. Therefore, it is unlikely that the Portuguese specimens originated as pelagic larvae transported by surface currents from the Western to the Eastern Atlantic.

Translocated species often have an anthropic origin. Ship ballast transportation and fish trade for aquaculture are the most common pathways for the introduction of alien marine species (Molnar *et al*., 2008; Katsanevakis *et al*., 2013). Among marine fish, one of the most striking examples is probably the introduction of the lionfish, *Pterois volitans* (Linnaeus, 1758) in the western Tropical Atlantic (Lasso-Alcalá and Posada, 2010; Betancur-R. *et al*., 2011; Green *et al*., 2012). These accidental escapes can cause damaging ecological impacts (Naylor *et al*., 2001; Simberloff *et al*., 2013). However, despite knowing the potential problems and that the eradication or removal of established invaders have a low probability of success (Thresher and Kuris, 2004), the introduction of alien species for aquaculture is still promoted in many countries. The accidental release of fish from floating cages could explain the presence of similar sized individuals here. But as far as we know, no intention to farm *C. regalis*, which is not a main cultured species in North America or elsewhere, has been reported in Portugal. A release from the aquarium trade is also unlikely since aquarists do not value this species.

Shipping has already been advocated for several occasional occurrences of Western Atlantic species in European waters: e.g. *Pinguipes brasilianus* by Orsi-Relini (2002), *Micropogonias undulatus* by Stevens *et al*. (2004) and *Kyphosus incisor* by Orsi-Relini *et al*. (2010). However, these records were of isolated individuals. The proximity of the Port of Sines, the largest in Portugal, makes it a possible site for the appearance of exotic marine species introduced with the ballast water of merchant ships. The Port of Setubal allows the entry of large vessels, and the connection with the Sado River makes this natural bay a favourable oligohaline environment for the survival of *C. regalis* juveniles. However, the number of specimens observed in this study might indicate an already well established and disseminating population of *C. regalis,* resulting from the transport of large numbers of eggs or larvae in ballast waters.

The specimen observed by Acosta *et al*. (2013) was erroneously ascribed to *C. nebulosus*, but clearly belongs to *C. regalis*. To what extent these specimens from a similar area of the Iberian Peninsula might be related deserves clarification.

CONCLUSION

For the moment, *Cynoscion regalis* must be considered as a newcomer to the Eastern Atlantic as no evidence of a self-sustaining population currently exists. However, the mechanisms, through which this alien species was introduced into Portugal, need to be studied in order to prevent further introductions. We would also recommend the monitoring of the evolution of Setubal population, and an investigation into a possible connection with the Guadalquivir estuary.

Acknowledgements. – The authors are grateful to the fishermen of Setubal who kindly donated their fish for our study. Thanks are due to Gaël Piqués who informed us that he observed the same fish at the Port of Setubal fish market. We also thank the Service de Systématique Moléculaire (UMS 2700, part of the Plateforme Analytique du Muséum national d'Histoire naturelle) for granting access to its molecular platform, Céline Bonillo for her technical support and Jill Cucchi for copy-editing.

REFERENCES

ACOSTA J.J., CANOURA J. & JUÁREZ A., 2013. - First record of *Cynoscion nebulosus* in the Spanish waters of the Gulf of Cadiz (ICES Division IXa South). *Mar. Biodiv. Rec*., 6: e112.

BETANCUR-R. R., HINES A., ACERO-P. A., ORTÍ G., WILBUR A.E. & FRESHWATER D.W., 2011. - Reconstructing the lionfish invasion: insights into Greater Caribbean biogeography. *J. Biogeogr*., 38: 1281-1293.

CARNEIRO M., MARTINS R., LANDI M. & COSTA F.O., 2014. - Updated checklist of marine fishes (Chordata: Craniata) from Portugal and the proposed extension of the Portuguese continental shelf. *Eur. J. Taxon*., 73: 1-73.

CHAO N.L., 1986. - Sciaenidae. *In* Fishes of the North-eastern Atlantic and the Mediterranean (Whitehead P.J.P., Bauchot M.L., Hureau J.C., Nielsen J. & Tortonese E., eds), Vol. II, pp. 865-874. Paris: Unesco.

CHAO N.L., 2003. - Taxonomy of the seatrout, genus *Cynoscion* (Pisces, Sciaenidae), with artificial keys to the species. *In* Biology of the spotted seatrout (Bortone S.A., ed.), pp. 5-15. Boca Raton: CRC Press.

CHAO N.L., FRÉDOU F.L., HAIMOVICI M., PERES M.B., POLIDORO B., RASEIRA M., SUBIRÁ R. & CARPENTER K., 2015. - A popular and potentially sustainable fishery resource under pressure-extinction risk and conservation of Brazilian Sciaenidae (Teleostei: Perciformes). *Glob. Ecol. Conserv*., 4: 117-126.

GOLANI D., ORSI-RELINI L., MASSUTÍ E., QUIGNARD J.P. & DULCIC J., 2007. - Fish invasion of the Mediterranean – retrospective and prospective. *Rapp. Comm. Int. Mer Médit*., 38: 10.

GOLANI D., SONIN O. & RUBINSTEIN G., 2015. - Records of *Paralichthys lethostigma* and *Sciaenops ocellatus* in the Mediterranean and *Channa micropeltes* in Lake Kinneret (Sea of Galilee), Israel. *Mar. Biodiv. Rec*., 8: e39.

GREEN S.J., AKINS J.L., MALJKOVIC A. & CÔTÉ I.M., 2012. - Invasive lionfish drive Atlantic coral reef fish declines. *PLoS ONE*, 7(3): e32596.

HINSINGER D., DEBRUYNE R., THOMAS M., DENYS G., MENESSON M., GALLUT C., UTGE J. & DETTAI A., 2015. - Fishing for barcodes in the Torrent: from CO1 to complete mitogenomes on NGS platforms. *DNA Barcodes,* 3: 170-186.

IWASAKI W., FUKUNAGA T., ISAGOZAWA R. *et al*. [11 authors], 2013. - MitoFish and MitoAnnotator: a mitochondrial genome database of fish with an accurate and automatic annotation pipeline. *Mol. Biol. Evol.,* 30(11): 2531-2540.

KATSANEVAKIS S., ZENETOS A., BELCHIOR C. & CARDOSO A.C., 2013. - Invading European Seas: Assessing pathways of introduction of marine aliens. *Ocean Coast. Manage*., 76: 64-74.

LASSO-ALCALÁ O. & POSADA J.M., 2010. - Presence of the invasive red lionfish, *Pterois volitans* (Linnaeus, 1758), on the coast of Venezuela, southeastern Caribbean Sea. *Aquat. Invasions*, 5: S53-S59.

MIYA M. & NISHIDA M., 2015. - The mitogenomic contributions to molecular phylogenetics and evolution of fishes: a 15-year retrospect. *Ichthyol. Res*., 62: 29-71.

MOLNAR J.L., GAMBOA R.L., REVENGA C. & SPALDING M.D., 2008. - Assessing the global threat of invasive species to marine biodiversity. *Front. Ecol. Environ.*, 6: 485-492.

NAYLOR R.L., WILLIAMS S.L. & STRONG D.R., 2001. - Aquaculture – A gateway for exotic species. *Science*, 294(5547): 1655-1656.

ORSI-RELINI L., 2002. - The occurrence of the south American fish *Pinguipes brasilianus*, Cuvier and Valenciennes, 1829, in the Mediterranean. *Cybium*, 26: 147-149.

ORSI-RELINI L., COSTA M.R. & RELINI M., 2010. - First record of the yellow sea chub, *Kyphosus incisor*, in the Mediterranean. *Mar. Biodiv. Rec.*, 3: e4.

SASAKI K., 1989. - Phylogeny of the family Sciaenidae, with notes on its zoogeography (Teleostei, Perciformes). *Mem. Fac. Fish., Hokkaido Univ.*, 36(1): 1-137.

SHEPHERD G.R. & GRIMES C.B., 1984. - Reproduction of weakfish, *Cynoscion regalis*, in the New York bight and evidence for geographically specific life history characteristics. *Fish. Bull.*, 82(3): 501-511.

SIMBERLOFF D., MARTIN J.L., GENOVESI P. *et al.* [14 authors], 2013. - Impacts of biological invasions: what's what and the way forward. *Trends Ecol. Evol.*, 28: 58-66.

STEVENS M., RAPPÉ G., MAES J., VAN ASTEN B. & OLLEVIER F., 2004. - *Micropogonias undulatus* (L.), another exotic arrival in European waters. *J. Fish Biol.*, 64: 1143-1146.

STROHM J.H.T., GWIAZDOWSKI R.A. & HANNER R., 2015. - Mitogenome metadata: current trends and proposed standards. *Mitochondrial DNA*, [Epub ahead of print, Sep 4: 1-7]. DOI: 10.3109/19401736.2015.1015003

THRESHER R.E. & KURIS A.M., 2004. - Options for managing invasive marine species. *Biol. Invasions*, 6: 295-300.

TREWAVAS E., 1979. - Sciaenidae. *In*: Check-list of the fishes of the north-eastern Atlantic and of the Mediterranean. Vol. I (Hureau J.C. & Monod T., eds), pp. 58-69. Paris: Unesco.

WELSH W.W. & BREDER C.M. Jr., 1924. - Contributions to the life histories of Sciaenidae of the eastern United States coast. *Bull. U.S. Bur. Fish.*, 39: 141-201.

WINNEPENNINCKX B., BACKELJAU T. & DE WACHTER R., 1993. - Extraction of high molecular weight DNA from molluscs. *Trends Genet.*, 9: 407.